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BRIEF REPORT

Potassium nutrition in oil palm: The potential of metabolomics as a tool for precision agriculture

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Societal Impact Statement

Oil palm is the first oil-producing crop globally, representing nearly 20 million ha. In the recent past, oil palm cultivation has been controversial not only because of land utilisation at the expense of primary tropical forests or health concerns associated with palm oil, but also pollution caused by fertilization (including CO_2 produced to synthesise fertilizers). Oil palm fields are heavily fertilized with potassium (K), and thus finding better, more parsimonious methods to monitor K nutrition is more important than ever. Here, we suggest that metabolomics and subsequent machine learning of metabolic signatures represent a promising tool to probe K requirements, opening avenues for precision agriculture in oil palm industry.

KEYWORDS

diagnostic, machine-learning, metabolomics, oil palm, potassium

1 | POTASSIUM NUTRITION IS A MAJOR CONCERN FOR OIL PALM AGRICULTURE

Oil palm (*Elaeis guineensis* Jacq.) is the major oil-producing crop in the world, with a global annual production of about 75 Mt (FAO 2018). Low potassium (K) availability is a major concern on tropical soils where oil palm is cultivated since they are often naturally poor in exchangeable cations such as K^+ (Ollagnier & Ochs, 1973). In addition, oil palm growth is highly K-demanding. In effect, optimal leaflet K elemental content is quite high (≈1%) while N is about 3% (Foster, 2003; Ochs, 1965; Ollagnier et al., 1987) although there are some variations with seasons, locations and oil palm crosses (Foster & Chang, 1977; Ollagnier & Ochs, 1981). Also, fruit bunch harvesting removes substantial amounts of K from oil palm agrosystems. For example, typical fruit harvesting of 30 tons FFB (fresh fruit bunches) ha^{-1} y^{-1} represents a loss of up to 160 kg K/ha y^{-1}, that is, 75% of K fertilization input (reviewed in (Corley & Tinker, 2016)). Oil palm plantations are thus heavily fertilized with K (typically using potassium chloride, KCl) up to 200 kg K/ha y^{-1}, leading to an annual cost of about $1 billion at the global scale. However, the efficacy of applied K depends on leaching, the efficiency of K absorption by roots (including the antagonism between K and other cations, mostly Ca and Mg), K allocation within the tree and the response of yield to K availability in the variety (cross) of interest (Goh et al., 2003). Quite understandably, intense efforts have been devoted for decades to improve fertilization strategies and monitor K requirement accurately.

Presently, K fertilization is based on either agrosystem nutrient balance (Henson, 1999), leaf diagnostic (French method, which relies on critical %K in tissues (Caliman et al., 1994)), response coefficients of yield to applied K using differential decomposition (Foster's method, (Foster, 2003)), or calculation of K demand (Malaysian method or INFERS, (Kee et al., 1994)). However, these techniques rely on prior knowledge of oil palm response to K and thus typical quantities of fertilizer listed in reference tables. Therefore, the major problem is that these methods are semi-empirical and require systematic agronomical trials to document the response to K in the cross of interest, under the climatic conditions and soil type considered. That is, their implementation requires data from a large number of reliable multifactorial fertilization experiments. As such, it is very tedious and in principle lacks a mechanistic understanding of how K availability modulates growth, fruit production and ultimately, yield.

2 | IMPLEMENTATION OF METABOLOMICS IN OIL PALM SAPLINGS

To overcome this problem, the description of metabolic effects of K availability offers an excellent perspective, not only to understand how K nutrition controls oil palm physiology but also to find typical metabolic signatures that can be implemented via machine-learning to determine how much fertilization palm trees require. Recently, the potential of putrescine as a biomarker of K deficiency in relation to K, Mg and Ca balance has been described (Cui et al., 2020). In fact, metabolomics is an integrative technology offering practical advantages over other omics (such as transcriptomics): a low cost (typically $5–10 per sample), simple workflow, and algorithms to extract metabolic information, and no need to have the annotated genome sequence of the plant line of interest.

Here we examined whether the metabolic signature can be used to provide direct indications on the K status and how it relates to growth potential (experiments and analysis workflow summarized in Figure 1a). To do so, we took advantage of metabolomics data obtained recently (Cui et al., 2019, 2020), where oil palm saplings (aged ≈1 y) of a commercial cross *Deli* × *LaMé*, were grown in a greenhouse nursery under controlled K conditions and sampled at 10, 11, and 12.75 months. Oil palm saplings were cultivated under low (0.2 mM KCl in nutrient solution), medium (1 mM), high (4 mM) K, with or without waterlogging, from the 11th month (see Methods S1 for further details on experimental design). In the present context, waterlogging is particularly interesting since it is a common stress for oil palm plantations next to rivers or installed on peatlands. Waterlogging inhibits sap circulation, impacts on catabolism (like K availability) and thus interacts with K nutrition (Cui et al., 2020). Some low-K saplings were also subjected to K resupply (at 4 mM) for 1 or 2 weeks. In doing so, we thus had situations where K provision was continuous and situations where it was perturbed. That is, our dataset gathers oil palm saplings representative of different life itineraries. We carried out leaflet and rachis GC-MS metabolomics and ICP-OES ionomics to quantify metabolites and elements, respectively (data from Cui et al., 2019; Cui, Lamade, & Tcherkez, 2020) and deposited on Metabolome Express (Carroll et al., 2010) under the accession reference 2018 oil palm K). We then used the dataset to perform machine-learning multivariate statistics based on orthogonal projection on latent structures (OPLS) (further details on methods for metabolomics are provided in Methods S2).

We first looked at the relationship between leaflet K elemental content (in mg/g DW) and absolute growth rate (g DW month^{-1}) (Figure 1b). Here, the vegetative growth rate was the response variable because the present work deals with saplings, not adult trees. Despite a generally positive relationship, there was no simple correlation since as expected, it depended on both age (the older the higher the growth rate) and growth conditions (e.g., lower growth rate under waterlogging). Data points were comprised between sigmoid envelope curves, reflecting either a simple Michaelis–Menten dependence of growth rate with potassium (curvature $\alpha = 1$) or a cooperative behavior of internal K pools ($\alpha = 5$). In the latter case, K^+ compartmentalization within saplings was so that growth-effective K content in leaflet was observed beyond a threshold (of about 15 mg/g DW). From this point, multivariate statistics could be used with two methods, using the K content as either an input (predicting) variable X or an objective (response) variable Y. The former is useful to appreciate the importance of the role of K (amongst other metabolites and elements) for growth. The latter (in which elemental contents are withdrawn from the dataset to keep only metabolites) is the way to go to check whether leaflet metabolome is a good predictor of K and growth rate, and thus has some potential for utilization in nutritional monitoring.

The first method is illustrated in Figure 1c with a volcano plot that shows the weight of leaflet compounds in defining the component aligned with growth (and independent of age) generated by the OPLS (*y* axis), against the *p*-value obtained by classical univariate statistics using linear multiple regression (*x* axis). This representation is very convenient to locate most important biomarkers of growth at the right and left extremities of the volcano plot. Here we confirm that K (red arrow) is amongst major factors driving growth in addition to carbohydrates (such as glycerol 3-phosphate or fructose 6-phosphate). By contrast, other cations (Ca, Mg) are anti-correlated to growth, reflecting the well-known antagonism between K and other cations. However, it is worth noting that although K appears to be a strong driver of growth in the volcano plot (Figure 1c), K alone is not sufficient to infer the growth rate since similar mineral compositions (same K content) can be associated with very different growth rates (Figure 1b).

The output of the second method is shown in Figure 1d as a bi-plot that represents how growth and K content relate to components and what metabolites can explain them. The superimposition of samples and metabolites on the same graph allows one to locate their position in the multidimensional metabolic space and thus understand metabolites that explain response variables (growth, K content). The first component (*x* axis) was driven by both growth rate and K, and was closely related to age (time) and metabolites like sucrose (reflecting the photosynthetic input). The second component

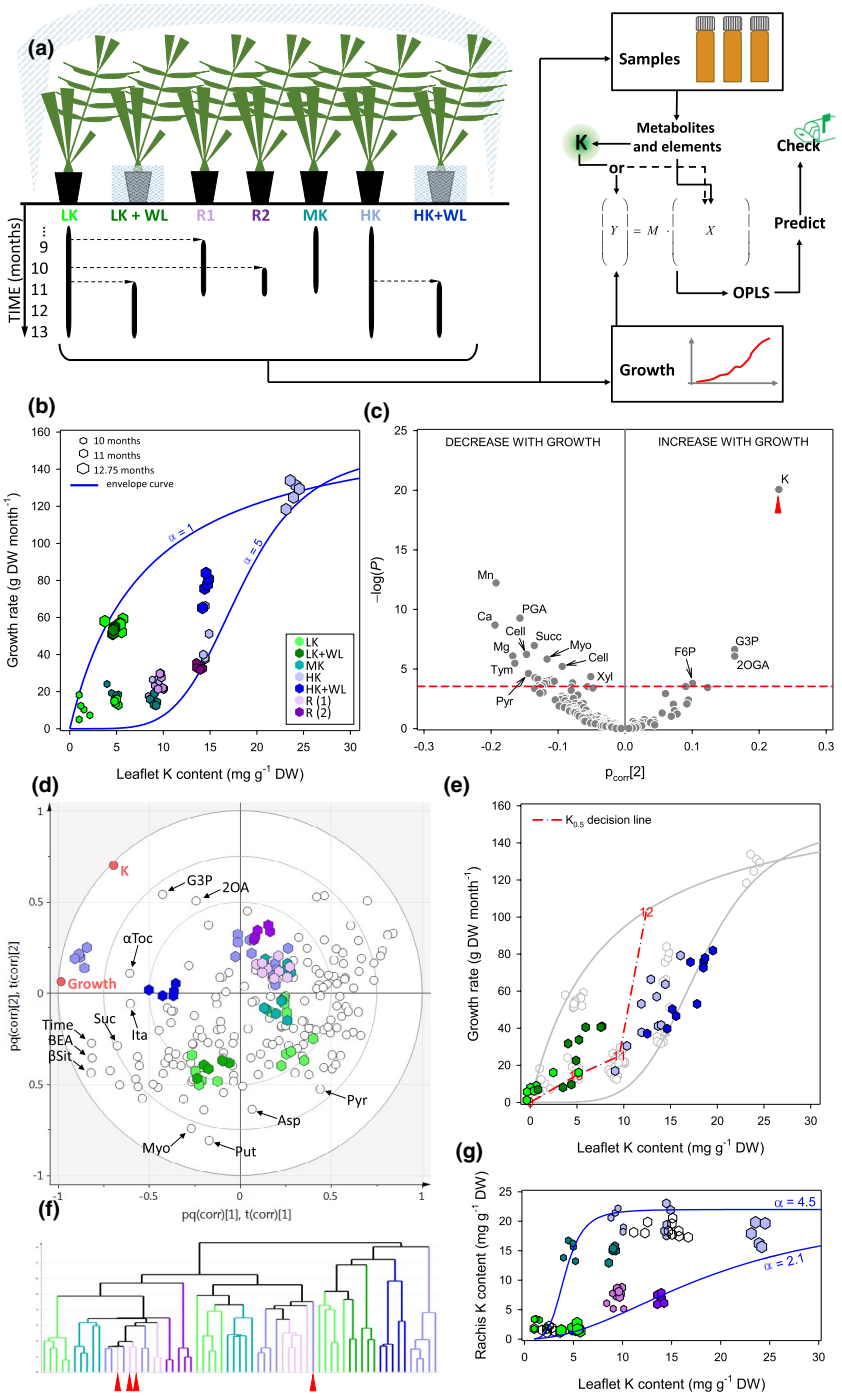

FIGURE 1 Machine-learning analysis of the relationship between potassium nutrition and leaflet metabolome in oil palm saplings *Deli* × *LaMé*. (a) Adopted experimental design and workflow. R1 and R2 refer to early and late K resupply, respectively. (b) Relationship between growth rate and potassium elemental content, with differentiation of age (symbol size) and K conditions (colors). (c) Volcano plot (–log(*p*-value) of univariate analysis against loadings in OPLS multivariate analysis, implemented with Simca®) showing best determinants of growth rate independent of tree age. *p*-values are associated with the relation with growth rate, using multiple linear regression of the form lm(Metabolite ~ Growth rate + Age) implemented in R. The dashed red line represent the Bonferroni significance threshold ($p = 10^{-3.54}$). (d) biplot showing the location of K, growth rate and samples in the metabolic multidimensional space. (e) Predicted growth rate and K content in extra samples, and their position with respect to the halfway (medium K) decision line. Samples used to draw the figure in panel (b) are recalled with empty symbols. (f) Hierarchical classification of samples using components (axes) of the multivariate analysis. (g) Relationship between leaflet and rachis K content. Data from 11.5-month-old palms (measured rachis K vs. predicted leaflet K) are shown with empty symbols. In both (b) and (g), blue lines stand for sigmoids with curvature (α) as indicated. Abbrevations: 2O(G)A, 2-oxoallonate; αToc, α-tocopherol; Asp, aspartate; BEA, benzene ethanamine; βSit, β-sitosterol; Cell, cellobiose (appears two times in the volcano plot since it generates two analytes during derivatization prior to GC-MS analyses); F6P, fructose 6-phosphate; G3P, glycerol 3-phosphate; Ita, itaconate; Myo, myoinositol; PGA, 3-phosphoglycerate; Put, putrescine; Pyr, pyroglutamate; Suc, sucrose; Succ, succinate; Tym, tyramine; Xyl, xylose

(*y* axis) was solely defined by K (the growth rate had nearly no coordinate on this axis). This agrees with the fact that the K content co-varied with growth but was also determined by other mechanisms independent of growth and age (Figure 1d). Such mechanisms manifested themselves by an increase in some metabolites (glycerol 3-phosphate and 2-oxoallonate for example) and a decrease in others (such as putrescine, aspartate, and myoinositol) when the K content increased. This response is consistent with documented effects of K availability on metabolic pathways in oil palm (Cui et al., 2019; Mirande-Ney et al., 2020). Taken as a whole, the statistical model was highly explicative ($R^2 = 0.97$), robust (the cross-validated R^2, denoted as Q^2, was 0.93), and highly significant ($p = 10^{-21}$ for K).

Waterlogging or "resupply" samples were then discarded from the dataset and used instead to test prediction capabilities. The metabolic signature appeared to be highly predictive, with an average error of 0.2–0.3 mg/g DW only for the K content and only about 2–8 g DW month^{-1} in growth rate (not shown). Extra samples from 11.5 month-old palms cultivated under low or high K, with or without waterlogging, were then used to test further the model and check whether it could locate samples in the correct region of the growth-K space. In fact, all samples appeared in the proper region. In addition, when a decision line was drawn halfway between low and high K, the model could identify the correct K status in 100% of cases (Figure 1e). Also, the metabolomics signature allowed proper classification of samples based on OPLS components, as only four out of 65 (i.e., 6%) samples were wrongly classified (red arrows), due to a confusion between two similar K-sufficient situations: resupply and high K oil palms (which were both under 4 mM KCl at the time of sampling) (Figure 1f).

The present analysis shown for leaflets in Figure 1a–f has also been carried out in rachis samples. Under our conditions, there was a less strong response of rachis K content to K availability under high K (plateauing, Figure 1g). Therefore, the multivariate analysis gave similar results although being slightly less predictive with rachis (not shown).

3 | PRESENT PERSPECTIVES OFFERED BY METABOLOMICS FOR K MONITORING

The present metabolomics tool was implemented in saplings and is thus relevant to plants grown in greenhouses and nurseries. As such, we used vegetative growth rate as a response variable. Future studies are warranted to test the performance of metabolomics in adult trees grown in plantation, with yield as a response variable. This will require a strict control of fertilization, soil composition and inter-tree heterogeneity, and will involve a work over several years. A recent analysis of metabolic properties of adult trees in the field has shown that 2 years are necessary to observe significant effects after the arrest of K fertilization (Mirande-Ney et al., 2020).

Still, our study shows that leaflet metabolome seems to be an excellent tool to monitor K nutrition in oil palm, allowing proper diagnostic in the growth-K space, which is essential to take fertilization decisions. Oil palm is currently a major oil-producing crop and its global cultivation area is 19 million ha (FAO 2018), representing about 2.5 billion trees. Oil palm cultivation is thus a strategic economic sector in tropical countries such as Indonesia or Malaysia, where its expansion must be sustainable. In particular, amongst criteria given by RSPO (Roundtable on Sustainable Palm Oil, www.rspo.org), fertilization must follow best agricultural practices including nutrient monitoring by sampling, avoidance of excessive inputs and nutrient recycling practices. Here new technologies such as metabolomics can play a role to provide optimal guidance for K fertilization.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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